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ORIGINAL PAPER

CONTENT OF SELECTED ESSENTIAL METALS IN THE MEAT OF PERCH (*PERCA FLUVIATILIS*) FROM NORTH-CENTRAL POLAND*

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Abstract

The aim of the present study was to analyze the impact of sex and localization on the levels of microelements (Cu, Zn and Fe) in the meat of perch (*Perca fluviatilis* L.) collected from the Bay of Puck (S1), the Vistula Lagoon (S2), the Vistula River (S3), the Drwęca River (S4) and the Włocławek Dam Reservior (S5). The study involved 83 sexually mature individuals of fish caught in autumn, divided into females and males, and having the body length in the range from 15.5 to 18 cm. The muscles samples were taken from the large side muscle of the fish body above the lateral line. Metal concentrations were determined by atomic absorption (AAS). The concentration of metals decreased in the following order: Zn > Fe > Cu and statistically significant differences were observed in the Zn, Fe and Cu content in the meat of fish collected from different water bodies. The mean content of Cu ranged from 2.97 mg kg^{-1} (S5) to 28.75 mg kg^{-1} (S2), Zn from 23.43 mg kg^{-1} (S3) to 53.09 mg kg^{-1} (S5) and Fe from 19.18 mg kg^{-1} (S3) to 38.38 mg kg^{-1} (S5). Sex was a factor differentiating the content of Cu in each sampling location. Correlation analyses confirmed statistically significant and negative correlations between Cu-Zn (r = -0.48) and between Cu-Fe (r = -0.51). The relationship between Zn and Fe was positive and statistically significant (r = 0.64). The sampling sites significantly affected metal-metal interactions. The research indicated that consumption of 100 g of perch meat could cover over 60% of daily requirement for Cu, and 13 and 7%, respectively, for Zn and Fe.

Keywords: microelements, sex, localization, perch, *Perca fluviatilis*.

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INTRODUCTION

Microelements such as Cu, Zn, Fe are responsible for the regulation of numerous metabolic pathways, so deficiencies in these nutrients are very dangerous and conducive to many medical conditions, i.e.: hypertension, anemia, demineralization of bones, rickets, water-electrolyte imbalance, inhibition of growth or disorders of the nervous system (Lidwin-Kaźmierkiewicz et al. 2009, Łuczyńska et al. 2009). Copper is a mcroelement which plays a significant role in the proper functioning of an organism, in the production of red blood cells and in the synthesis of RNA. Moreover, this metal is a cofactor of numerous enzymes participating in fatty acid metabolism, collagen synthesis and in the process of transportation and absorption of iron. It takes part in the processes of solidification of collagen, keratinization of hair and synthesis of melanin (Zhelyazkov et al. 2014). Zinc is an equally important microelement. Like copper, it plays an important role in the proper functioning of an organism, especially of the immune system. Moreover, it is a component of many metalloenzymes, it regulates metabolism of carbohydrates, proteins, nucleic acids, and participates in insulin synthesis and in bone mineralization (Yilmaz et al. 2010, Zubcov et al. 2012). Another very important microelement which we analyzed was iron, which is a cofactor of many enzymes and a component of chromoproteins of blood and muscles. In addition, iron is a metal which supports the proper functioning of the nervous and immune systems. Iron is also responsible for detoxification of harmful substances in the liver and is known to prevent anemia, as it is responsible for the production of red blood cells. Therefore, in response to the constant search for healthy food and natural products rich in minerals, it is worth recommending higher consumption of fishby humans. The annual intake of fish and fish products in Poland in 2016 was above 12 kg per person (Pieńkowska, Hryszko 2017) and intake of fish is *ca* 112 g per person per week, whereas the recommended weekly intake is *ca* 300 g per person (Winiarska-Mieczan et al. 2017). At the same time, properties of freshwater fish should not be neglected, including the so-called economically less valued species, i.e.: roach (*Rutilus rutilus*), white bream (*Blicca bjoerkna*), common bleak (*Alburnus alburnus*), ruffe (*Gymnocephalus cernua*) or perch (*Perca fluviatilis*). Perch meat enjoys a great and growing popularity among Polish consumers owing to its desirable traits, namely white colour, delicate texture and mild odor (Zakęś et al. 2003). It is well known that the level of minerals in fish meat is influenced by many different factors, i.e. the age of a fish, season of the year, fish species and type of tissue (Canli, Atli 2003, Farkas et al. 2003, Brucka-Jastrzębska, Protasowicki 2006, Klavins et al. 2009, Yilmaz et al. 2010, Stanek et al. 2016).

There are far fewer data on the impact of sex (Al-Yousuf et al. 2000, Zubcov et al. 2012, Järv et al. 2013, Rajkowska, Protasowicki 2013), but assuming that this factor may determine the level of metal accumulation in the body of fish, this study has been undertaken to explore the effect of sex on selected microelement levels (Cu, Zn and Fe) in the meat of females and males of perch collected from five biologically diverse water bodies (the Bay of Puck, Vistula Lagoon, the Vistula River (near Toruń), the Drwęca River and Włocławek Dam Reservior) were undertaken. Moreover, correlations between metals, and its percentage recommended daily intake (ZDS) for the analyzed minerals after consumption 100 g of perch meat (wet weight) were calculated. These studies could be a valuable source of an information for potential Polish consumers about the content of microelements in the popular fish species.

Study area

The Bay of Puck (Figure 1) is a branch of the Bay of Gdańsk in the Baltic Sea, separated from the open sea by the Hel Penisula. It is a eutrophic water body with a poor ecological and good chemical status, on which stands the harbour city of Gdynia (Report wioś 2017). Vistula Laggon – its Polish part (Figure 1) – is a lagoon of the Baltic Sea, having slightly salty water and

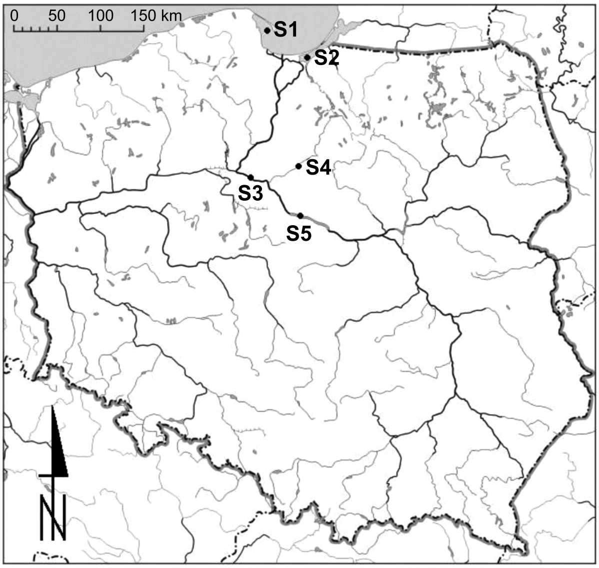

Fig.1. Map of Poland and localization of collected samples: S1 –Bay of Puck, S2 – Vistula Lagoon, S3 – Vistula River, S4 – Drwęca River, S5 – Włocławek Dam Reservoir

separated from Gdańsk Bay by the Vistula Spit. It belongs to the Natura 2000 network (the European Union network of protected areas) located in farmland area. This lagoon can be classified as a eutrophic water body and a stable ecosystem (Nawrocka, Kobos 2011, Kopiec 2015). Its distinguishing feature is the mixing of fresh and marine water. The sampling site on the Vistula River (Figure 1) was located in a nature protected area of the lower Vistula River, at a site at the height of the village Górski (near Toruń), where water monitoring showed a moderate ecological status (III class) (Szatten, Czerebiej 2018). The Drwęca River (Figure 1) flows through northern Poland and is a tributary of the Vistula River. This river belongs to the Natura 2000 network (Harnisz et al. 2015). The main sources of water pollution at the sites where fish were captured are waste discharged from the sewage treatment plant in Golub-Dobrzyń and agricultural runoffs. Włocławek Dam Reservoir (Figure 1) it is the second largest dammed reservoir in Poland, built in the 1970s, and situated at 657th km of the Vistula River; it is now a highly eutrophic water body (Mimier, Żbikowski 2017). The sampling site was located near Włocławek.

MATERIAL AND METHODS

Perch (*Perca fluviatilis* L.) was caught in autumn from five sites which were biologically extremely diverse (the Bay of Puck, Vistula Lagoon, the Vistula River – at the height of Górski, near Toruń, the Drwęca River and Włocławek Dam Reservior). Fish were caught using a fishing rod in accordance with the Directive of the Polish and the European Parliament and of the EU Council on the protection of animals used for scientific or educational purposes (*Directiv*e… 2010, *Polish Act*… 2015). The study was performed on 83 sexually mature fish, divided into females and males, with a body length in the range from 15.5 to 18 cm. Sex of the collected individuals was determined by the inspection of gonads after opening the body cavity. The muscle samples were taken from the large side muscle of the fish body above the lateral line and were frozen immediately after the preparation to be kept in a deep freezer until analyses. All frozen samples were freeze dried in a freeze drier (Finn-Aqua Lyovac GT2) (parameters: temp. -40°C, pressure $6 \cdot 10^{-2}$ mbar, duration at least 48 h).

The freeze dried samples were mineralized in a microwave mineralizer (Ethos Plus, Milestone). For mineralization, 0.1 g of the tissue was weighed and then a mixture of HNO_3 and H_2O_2 in the 4:1 ratio was added. During the first 10 min, the temp. was increased to 190°C. During the next 7 min the temp. was kept at 190 ± 5°C. The mineralized samples were transferred quantitatively to 50 cm^3 measuring flasks.

Cu, Zn and Fe concentrations were determined on a Thermo Elemental SOLAAR S2 Flame AA spectrophotometer, calibrated using Merck standard solutions (Merck KGaA). The validation of the analysis was conducted on certified standards – Certified Reference Material ERM®-BB422 Fish Muscle and Certified Reference Material BCR®-670 Aquatic Plant (Table 1). Tissue concentrations of minerals have been reported as mg kg^{-1} dry weight (mg kg^{-1} d.w.). The concentrations of the metals were calculated from linear calibration plots obtained from measurements of the standard solutions. All deter-

Table 1

Total content of elements in certified materials

Element	Certified value (mg kg^{-1})	Determined value (mg kg^{-1})	SD (%)
Cu	1.67 ± 0.16* 1.82 ± 0.30**	1.42 ± 0.24 1.63 ± 0.32	0.16 0.19
Zn	16.0 ± 1.1* 24.0 ± 2.1**	17.2 ± 1.20 25.2 ± 2.50	1.26 0.94
Fe	9.4 ± 1.4* 0.94 ± 0.07** (g kg^{-1})	10.1 ± 1.33 1.21 ± 0.20	1.21 2.24

* Certified Reference Material ERM®- BB422, ** Certified Reference Material BCR®-670

minations were made in triplicate and the data for samples of the meat were corrected to the oven-dry (105°C) moisture content.

Statistical analysis

Statistical analyses were carried out using Statistica 13.0 software (StatSoft 13.0). In order to assess normality of data distribution, the Shapiro-Wilk test was used, while the Levene's test was applied to analysis of homogeneity of variance. ANOVA, one-way analysis of variance (the Tukey's test), was used to test significant differences in the content of metals among the five sampling locations. The *t*-student test was used to analyse significant differences in the content of metals in the meat of females and males from all groups and from individual water bodies. The analyses of correlation between metal concentrations were made. The Pearson's rank correlation coefficient (*r*) was calculated.

RESULTS AND DISCUSSION

Statistical analyses indicated that metals accumulated in the meat of perch in the following order of magnitude: Zn > Fe > Cu in the most of locations, except S2 (Vistula Lagoon), where a higher content of Fe than that of Zn was observed (Figure 2, Table 2). The same results were reported by Łuczyńska et al. (2009) for perch from the Mazurian Great Lakes, Stanek et al. (2016) for perch caught in autumn in Lake Strzeszyńskie, Lake Wędromierz and Lake Góreckie (Western Poland) and by Yazdi et al. (2012) for perch from Anzali Wetland, Iran. The research has shown that Zn and Fe were determined in the largest amounts in the meat obtained from fish caught in Włocławek Dam Reservoir (S5) (53.09 and 38.38 mg kg^{-1}, respectively), which may have been due to the confirmed high level of eutrophication of this reservoir caused by anthropogenic pollution, including urban

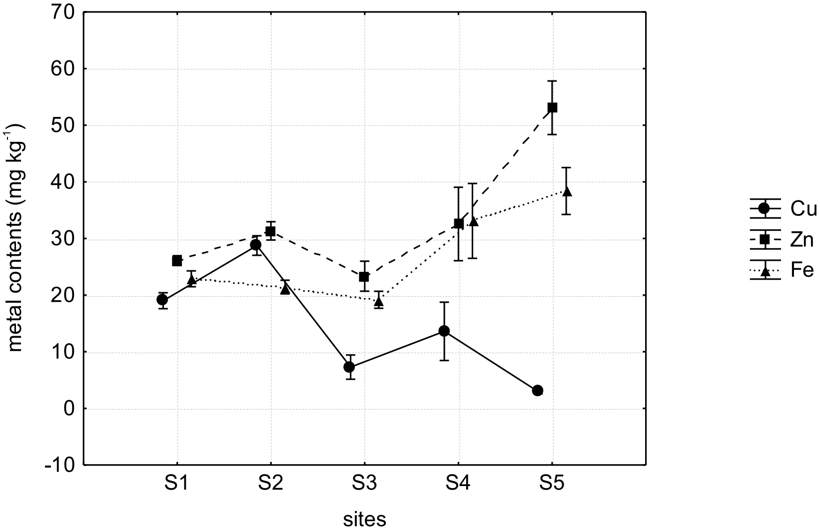

Fig. 2. The mean content of microelements (mg kg-1) in the meat of perch (*Perca fluviatilis*) collected from five sampling locations: S1 – Bay of Puck, S2 – Vistula Lagoon, S3 – Vistula River, S4 – Drwęca River, S5 – Włocławek Dam Reservoir

Table 2

Cu, Zn and Fe content (mg kg^{-1} d.w.) in the meat of perch (*Perca fluviatilis*) collected from five water bodies

Reservoir	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})
Bay of Puck (S1)	19.01 ± 2.99^{a}	26.10 ± 1.84^{a,b}	22.88 ± 2.99^{a}
Vistula Lagoon (S2)	28.75 ± 3.47^{b}	31.34 ± 3.24^{a}	21.42 ± 2.42^{a}
Vistula River (S3)	7.29 ± 3.70^{c}	23.34 ± 4.60^{b}	19.18 ± 2.59^{a}
Drwęca River (S4)	13.61 ± 7.20^{d}	32.56 ± 9.06^{a}	33.10 ± 9.23^{b}
Włocławek Dam Res. (S5)	2.97 ± 0.89^{e}	53.09 ± 10.39^{c}	38.38 ± 9.09^{b}

Values marked with the different letters in the same column are significantly different ($p < 0.05$, Tukey's test).

runoffs and seepage of nutrients from agriculture. A possible source of iron is decomposing organic substance. The meat of perch collected from Vistula Lagoon (S2) turned out to be the richest source of Cu (28.75 mg kg^{-1}). The main source of this metal may be wastewater from sewage treatment plants or plant protection products, because more than half of the area is under agricultural use and not much industry is developed there (KORNIJÓW 2018).

Levels of Cu, Zn and Fe obtained in the meat of the analyzed perch were higher than in the tissues of the same species caught in June in the Mazurian Great Lakes (northeastern Poland), in which the average content of those minerals ranged from 0.7 to 1.2, from 4.6 to 6.8 and from 20.2 to 24.5 mg kg^{-1} dw, respectively (Łuczyńska et al. 2009). Concentrations of Cu and Zn in the analyzed perch from the Vistula and Drwęca Rivers were in a very similar range as determined in the perch from Anzali Wetland (Iran) (from 7.30 to 13.00 mg kg^{-1} dw for Cu and from 16.45 to 37.05 mg kg^{-1} dw for Zn) (Yazdi et al. 2012). Similar values of Cu (0.08-0.36 mg kg^{-1} ww) and for Zn (4.41-8.48 mg kg^{-1} ww) were determined in the meat of perch form Vistula Lagoon analyzed by Polak-Juszczak (2003).

Analyses indicated statistically significant differences between females and males (in all groups of fish) in the mean content of metals (mg kg^{-1}) only for copper (Table 3). No statistically significant differences in the content of

Table 3

Differences in the metal content between females and males of perch (*Perca fluviatilis*) collected from five water bodies

Sex	*n*	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})
♂	42	12.06 ± 9.57^{a}	35.40 ± 14.46^{a}	26.72 ± 11.00^{a}
♀	41	16.65 ± 10.48^{b}	33.43 ± 11.76^{a}	27.44 ± 8.29^{a}

Values marked with the different letter in the same column are significantly different ($p < 0.05$, *t*-student test).

metals between females and males were achieved with respect to Zn and Fe. The same results concerning the mean content of zinc in female and male perch collected from the Barycz River were obtained by Marcinkowska and Dobicki (2014). Concentrations of Cu and Fe were higher in females of analyzed perch, but the meat of males contained more Zn. Analyses of carp (*C. carpio*) carried out by Alhashemi et al. (2012) indicated a very similar result regarding Zn in fish fillets (about 30 mg kg^{-1} dw), but females were richer in this mineral. More detailed analyses concerning differences in the microelement content between females and males that took into account individual sampling locations (t-test) showed statistically significant differences for Cu in all reservoirs, for Zn in the meat of fish caught from Vistula Lagoon (S2) and the Drwęca River (S4); as for Fe statistically significant differences ocurred in the case of the Bay of Puck (S1) and the Drwęca River (S4) – Figure 3. These results confirmed that the location of a water body is an important factor determining the degree of element accumulation between females and males.

Differences in the metal content between individuals of different sexes were observed for ruffe from the Vistula River (Stanek et al. 2017), for *Lethrinus lentjan* (Al-Yousuf et al. 2000) and for immature individuals of perch caught from the Baltic Sea (Järv et al. 2013). This tendency may result from certain disparity between males and females in the degree of membrane permeability, differences in the immune system development, the content of enzymes and hormones, and in the degree of binding metals and

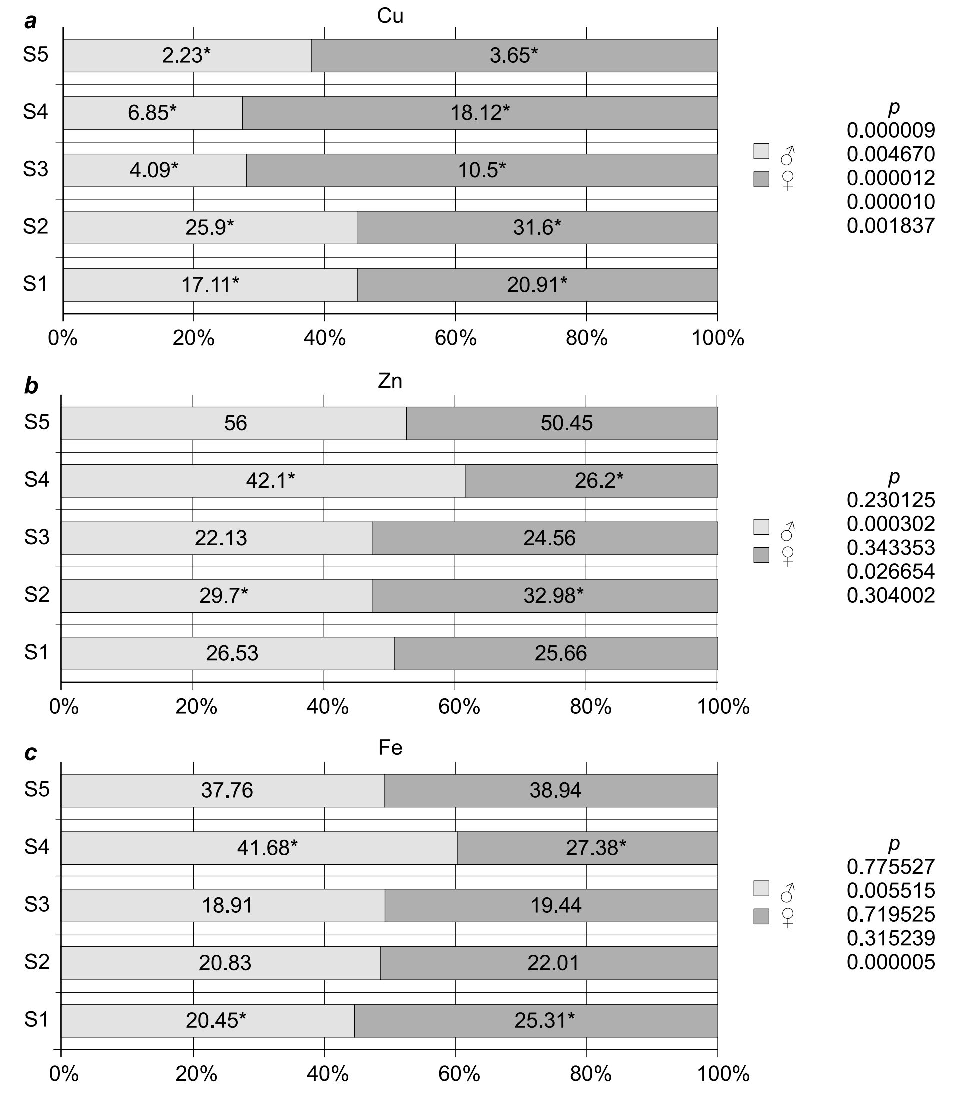

Fig. 3. Metal content ratios (males/females) of Cu – *a*, Zn – *b* and Fe – *c* (mg kg^{-1}) in the meat of perch (*Perca fluviatilis*) collected from five water bodies: S1 – Bay of Puck, S2 – Vistula Lagoon, S3 – Vistula River, S4 – Drwęca River, S5 – Włocławek Dam Reservoir (* statistically significant differences, $p < 0.05$)

their detoxification (Järv et al. 2013, Rajkowska, Protasowicki 2013). Gender differences may be explained by the fact that females are characterized by a faster growth rate, and comparison of males and females of the same body length indicates that females are younger than males. Moreover, differences in the metal concentrations between females and males are higher in immature individuals due to a faster metabolic rate, and decrease in adults (Järv et al. 2013). Higher content of Cu in the meat of females (in all reservoirs), Zn (in locations S2 and S3) and Fe (in locations S1, S2, S3 and S5) than in males confirms that younger individuals may bioaccumulate larger amounts of metals as they invest more energy in growth, food intake, and in tissue development than in the processes of detoxification. Therefore, mechanisms of metal excretion from the body are not sufficiently developed (Kljakovič Gašpić 2002, Dobicki, Polechoński 2003, Drąg-Kozak et al. 2011). Moreover, in the early fish growth stage, there is an increased amount of water filtered by gills, which may increase the amount of absorbed metals (Merciai et al. 2014) and on the other hand, an increased level of excretion of metals through gills, skin and the mouth and greater dilution of metals along with a growing fish body, that is increasing body volume in older individuals (Canli, Atli, 2003, Farkas et al. 2003),

Analyses of metal-metal correlations confirmed statistically significant and negative correlations between Cu and Zn (r = -0,48) and between Cu-Fe (r = -0,51). Relationship between Zn and Fe was positive and statistically significant (r = 0,64) – Table 4. The negative correlation between Cu-Zn con-

Table 4

Values of the Pearson's rank correlations coefficient (r) for selected metals in the meat of perch (*Perca fluviatilis* L.) in males and females

Zn	-0.48*			Zn	-0.48*			Zn	-0.49*	
Fe	-0.51*	0.64*		Fe	-0.51*	0.71*		Fe	-0.58*	0.56*
	Cu	Zn			Cu	Zn			Cu	Zn
All group				males				females		

* statistically significant differences, $p < 0.05$

firms the reduced Zn absorption process under the influence of Cu (Bajc et al. 2016). The sex of fish did not affect differences in the correlations between metals and values of correlation coefficients for males (a) and females (b) were similar (Table 4). The same results of correlation analyses between Fe and Zn (r = 0.56) were observed by Klavins et al. (2009) in the case of perch caught from inland waters in Latvia, but correlation between Cu and Fe was positive and statistically significant (r = 0.70). Positive and statistically significant correlation between Cu and Zn was calculated in the meat of perch from the southern Baltic (Szefer et al. 2003).

It is a very difficult to compare an inter-elemental relationships for one fish species living in a different reservoirs, because the rate at which metals may affect the organism could be related to the many factors, such as biologicalones, e.g. blood as a metal transporting factor, growing rate of the species, swimming activity, types of the analyzed tissues and environmental factors, i.e. differences in the state of an aquatic environment, changes in water temperature (Canli, Atli 2003, Brucka-Jastrzębska, Protasowicki 2006, Yilmaz et al. 2010). Correlation analyses taking into account the sampling location confirmed (in contrast to the sex) that the location of the reservoir was

Table 5

Values of the Pearson's rank correlations coefficient (r) for selected metals in the meat of perch (*Perca fluviatilis* L.) in five sampling locations: S1, S2, S3, S4 and S5

Zn	-0.44*			Zn	-0.50*			Zn	0.19	
Fe	-0.64*	-0.37		Fe	-0.37	-0.04		Fe	0.23	-0.07
	Cu	Zn			Cu	Zn			Cu	Zn
S1				S2				S3		
Zn	-0.78*			Zn	-0.20					
Fe	-0.79*	0.94		Fe	-0.09	-0.31				
	Cu	Zn			Cu	Zn				
S4				S5						

* statistically significant differences, $p < 0.05$

Table 6

The content of minerals in the meat of analyzed fish, recommended daily intake (RDI) of minerals and % RDI for 100 g of fish meat

Metals	RDI* (mg)	Content in the perch meat (mg 100 g^{-1} w.w.)	%RDI for 100 g of meat
Cu	0.9	0.059 - 0.575	6.60 - 63.89
Zn	8 - 11	0.467 - 1.062	5.83 - 13.27
Fe	10	0.384 - 0.768	3.84 - 7.68

* RDI – recommended daily intake according to nutrition standards developed by the National Food and Nutrition Institute in Warsaw (Jarosz 2017).

a significant factor determining the metal-metal interactions. The most statistically significant coefficients were calculated in the meat of fish from the Drwęca River (S4) and this results is worth further investigations (Table 5).

Table 6 presents the content of the microelements determined in the meat of perch calculated per 100 g of wet weight (taking into account the percentage of water in the tissue of 80%) and a % of the recommended daily intake (RDI) for the analyzed minerals. The research indicated that consumption of 100 g of perch meat could satisfy over 60% of the daily requirement for Cu, and 13 and 7%, respectively, for Zn and Fe. Moreover, our analyses showed that in no case did the metal content surpass the Maximum Permissible Limit (MPL) in fish meat recommended by WHO, FAO and EC, which is 30 μg g^{-1} w.w. for Cu, 30-100 μg g^{-1} w.w. for Zn and 100 μg g^{-1} w.w. for Fe (El-Moselhy et al. 2014). Analyses of perch from West Pomeranian (Poland) carried out by Lidwin-Kaźmierkiewicz et al. (2009), showed that a 100 g edible portion of meat would satisfy ca 1%, 2.6% and 1.5% of human daily demand for Cu, Zn and Fe, respectively.

CONCLUSIONS

In summary, the conducted analyses confirmed that the site of collecting fish was the most important factor influencing the degree of accumulation of analyzed micronutrients in meat and inter-elemental relationships. The meat of fish collected from Włocławek Dam Reservoir was revealed to be the richest source of Zn and Fe. The largest amount of Cu was determined in perch from Vistula Lagoon. Our analyses verified that the sex of perch was an important factor differentiating the levels of metals in the meat.

Taking into account the recommended daily intake (RDI) for macroelements, our analyses demonstrated that perch meat may be a valuable source of minerals important for the proper functioning of a human organism. Consumption of 100 g of perch meat could satisfy over 60% of the daily requirement, 13% for Zn and 7% for Fe.

Conflicts of interest

All authors declare that they do not have any financial competing interests or other conflicts of interest related to the current manuscript.

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